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EXAMINER	
RAO, MANJUNATH N	

ART UNIT	PAPER NUMBER
1652	8

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/542,121	RUCH, FRANK	
	Examiner	Art Unit	
	Manjunath N Rao	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 January 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-36* is/are pending in the application.

4a) Of the above claim(s) *2,14-24 and 31-36* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-13 and 25-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *04 April 2000* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *4, 5,* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claims 1-36 are currently pending in this application. Claims 1, 3-13, 25-30 are now under consideration. Claims 2, 14-24, 31-36 have been withdrawn from consideration as being drawn to non-elected invention.

Election/Restrictions

Applicant's election with traverse of Group I, Claims 1, 3-13, 25-30 in Paper No. 7 is acknowledged. The traversal is on the ground(s) that coexamination of all of Groups I-III would not be an undue burden on the Examiner. This is not found persuasive because while the searches for the three groups overlap, they are not coextensive. The search for Groups II and III would each require the search of subclasses unnecessary for the search of elected Group I. For example, search of Group II would require search of subclass 426/42 and search of Group III would require search of subclass 424/93.2

The requirement is still deemed proper and is therefore made FINAL.

Claims 2, 14-24, 31-36 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention, the requirement having been traversed in Paper No. 7.

Priority

Applicant's claim for domestic priority under 35 U.S.C. 119(e) is acknowledged.

Drawings

This application contains drawings that have been accepted by the Examiner for examination purposes only.

Claim Objections

Claims 3 and 26 are objected to because of the following informalities: Claims 3 and 26 recites the names of two bacteria *Bifidobacteria* and *Leuconostoc* with incorrect spellings. Claims 3 and 26 also recite the name of the genus *Streptococcus* twice, once in line 2 and once in line 4. Appropriate correction is required.

Claim 10 is objected to because of the following informalities: Claim 10 is objected because it is unclear to the Examiner as to, whether the agents for permeabilization are claimed simply in the alternative or as a Markush group. If the applicants intended to claim the permeabilzing agents as a Markush group then replacing the word "or" with "and" in line 2 would overcome this objection.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 12 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 12 recites the limitation "detergent" in line 1. There is insufficient antecedent basis for this limitation in the claim. It appears that applicants intended claim 12 to depend from claim 10. If this is so, amending the claim appropriately would overcome this rejection.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1 and 3-5, 10-13, 25-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Somkuti(a) et al. (Enzyme Microb. Technol., 1994, Vol. 16:573-576), Somkuti(b) et al. (Curr. Microbiol., 1998, Vol. 36:202-206), Herman et al. (In Streptococcal Genetics, Ferrretti and Curtiss(Eds), pages 225-228, 1987), VanBelkum et al. (J. Bacteriol., 1991, Vol. 173(24):7934-7941), Lee et al. (Biotechnol. Bioeng., 1996, Vol. 52(5):572-578) and Chang et al. (US 5,766,907, 6-16-98). Claims 1 and 3-5, 10-13, 25-30 in this instant application are drawn to a method for preparing a lactase microcarrier wherein the method comprises transforming a food-grade lactic acid bacterium such as *L.lactis* with a DNA construct comprising a promoter operatively linked to a DNA sequence encoding a beta-galactosidase (BG) such as the beta-galactosidase of *S.thermophilus*, culturing the bacterium under conditions that enable expression of the BG such that the bacterium exhibits a BG activity of at least 4,000-10,000 Miller Units (MU) and permeabilizing the bacterium by an agent such as a chemical, a solvent such as ethanol or a detergent such as deoxycholate or SDS followed by obtaining the permeabilized bacterium as a composition, cell suspension, or as immobilized cells.

Somkuti(a)et al. teach permeabilized *S.thermophilus* for the preparation of low lactose milk which involves the hydrolysis of lactose in milk. The reference also teaches that *S.thermophilus* is an organism that is extensively used in the production of yogurt, cheese and

that its BG is extensively studied in detail. The reference teaches that *S.thermophilus* has also been suggested as a source of BG suitable for reducing the lactose content of milk which is especially useful for people with lactose intolerance. While elaborating all the above characteristics of the microorganism, the reference also teaches the advantages of permeabilization and provides methods for permeabilizing the cells using detergents such as SDS, triton etc. The reference also teaches lactose hydrolysis in permeabilized cells. However, *S.thermophilus* is considered as food-grade lactic acid bacteria (LAB) the reference does not teach the use of LAB such as *L.lactis* or the use of ethanol for permeabilization or the use of recombinant BG gene such as the BG gene from *S.thermophilus*, under the control of a promoter or that the BG produced by the permeabilized cells are of the order of 4,000-10,000 MU.

Somkuti (b) et al. teach the permeabilization of *S.thermophilus* and *L.delbrueckii* using ethanol. The reference teaches that there is a concern in the industry that lactic acid bacterial cultures permeabilized using solvents or detergents might carry over residues of the same which may end up in the finished products. The reference teaches in that regard, ethanol as an ideal solvent of choice since it is present in trace amounts of many fermented dairy products consumed by humans and that ethanol has long been used to permeabilize yeasts. The reference however does not teach the use of *L.lactis* for the above purpose or the use of recombinant BG gene such as the BG gene from *S.thermophilus* under the control of a promoter or that the BG produced by the permeabilized cells are of the order of 4,000-10,000 MU.

VanBelkum et al. teach that lactococcin A, a bacteriocin produced by *L.lactis* increases the permeability of the cell membrane of *L.lactis*. The reference, however does not teach the

use of *L.lactis* for recombinant BG production up to the order of 4,000-10,000 MU under the control of a promoter.

Herman et al. teach the characterization of plasmids and cloning of beta-galactosidase gene from *S.thermophilus*. Again this reference also does not teach the use of the cDNA for recombinant BG gene under the control of a promoter or that the BG produced by the permeabilized cells are of the order of 4,000-10,000 MU.

Lee et al. teach the production of very high levels of beta-galactosidase (up to 36,000 MU) in an *E.coli* transformed with a plasmid comprising a beta-galactosidase gene under the control of "nar" promoter. The reference teaches methods to construct the vector and methods to induce the expression of beta-galactosidase in transformants. However, the reference does not teach the same using a lactic acid bacterium such as *L.lactis* or the use of recombinant BG from *S.thermophilus* for the above purpose. The reference also does not teach the permeabilization of the cells.

Chang et al. teach a method for immobilization of whole microbial cells containing in Ca-alginate capsules. The referene provides a liquid cored sphrical capsule that provides more extensive volume for microbial growth which enables a highly concentrated culture of microbes therein. The reference also teaches that immobilized cells have a wide range of applicability including the use of the microbial cell as a source of enzyme required for specific enzymatic reactions while maintaining high concentrations of cells within a capsule.

Thus it appears that , the permeabilization of lactic acid bacteria such as *S.thermophilus* was well known in the art and the use of such cells for production of milk and milk products aimed towards the use by lactose intolerant people was also well known in the art. It also

appears that the use of agents such as detergents and ethanol was also well known in the art for permeabilization of lactic acid bacteria. The art also teaches the cDNA clone for *S.thermophilus* BG and that the enzyme from *S.thermophilus* was most favored in the industry. It also appears that there has been several attempts in the art to produce BG at high levels for using it for different purposes. This is clearly evident from the reference of Lee et al. who demonstrate the production of up to 36,000 MU of BG enzyme.

It would have been, obvious to one of ordinary skill in the art, especially those interested in developing lactic acid bacteria, in view of its food-grade status, to develop a method for producing them as microcarrier for BG enzyme. While permeabilizing methods were known, as Somkuti et al. teach, it would have been obvious to one of ordinary skill in the art to use a detergent or ethanol (to avoid the residual effects of the detergent) to permeabilize the lactic acid bacteria. Combining the references of Somkuti et al. with that of VanBelkum et al. it would have been obvious to one of ordinary skill in the art to use *L.lactis* for permeabilization and introducing the cDNA encoding BG as it produces lactococcin A which is known to increase permeability of the cells which would be an added advantage to permeabilization by ethanol. It would have been obvious to combine the reference of Herman et al. with the above references and use the cDNA clone provided by Herman et al. to transform *L.lactis* of VanBelkum to obtain a recombinant *L.lactis* with a BG gene from *S.thermophilus* which is most preferred by the industry. It would have been further obvious to combine the reference of Lee et al. with all the above references to make a construct comprising the BG gene from *S.thermophilus* ---because of its ability to be active at slightly elevated temperatures-- operatively linked to "nar" promoter and use such a vector to transform *L.lactis* such that the resulting recombinant *L.lactis* produced

very high levels of thermophilic BG anywhere from 4,000 to more than 10,000 MU. Using such a transformed *L.lactis* it would have been obvious to permeabilize it either using the detergents or ethanol as taught by Somkuti et al. to obtain a lactic acid bacterial strain which is capable of producing very high levels of BG with high permeability such that it would be an ideal product as a microcarrier for BG to hydrolyze lactose in milk and milk products. Next, it would have been obvious to one of ordinary skill in the art to combine all the above references with that of Chang et al. to package the highly permeable, and high BG producing *L.lactis* in immobilized form using calcium alginate method such that the recombinant bacteria can be transported and stored to be used where and when required. One of ordinary skill in the art would have been motivated to do the above since there is a demand for agents which can be ingested safely and at the same time hydrolyze lactose in milk and milk products by lactose intolerant people and also due to the fact that the *S.thermophilus* enzyme, with its relatively high (50-55 degree C) optimum temperature could replace less-heat-tolerant beta-galactosidase preparations derived from yeasts that are currently used on a commercial scale. One of ordinary skill in the art would have had a reasonable expectation of success since all the above references have demonstrated partially each part of the invention separately.

Therefore the above invention would have been *prima facie* obvious to one of ordinary skill in the art.

Claims 6-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Somkuti(a) et al. (Enzyme Microb. Technol., 1994, Vol. 16:573-576), Somkuti(b) et al. (Curr. Microbiol., 1998, Vol. 36:202-206), Herman et al. (In Streptococcal Genetics, Ferrretti and Curtiss(Eds),

pages 225-228, 1987), VanBelkum et al. (J. Bacteriol., 1991, Vol. 173(24):7934-7941) and Lee et al. (Biotechnol. Bioeng., 1996, Vol. 52(5):572-578) as applied to claims 1 and 3-5, 10-13, 25-30 above, and further in view of Kuipers et al. (US 5,914,248, 6-22-99). Claims 6-9 are drawn to the use of a promoter for expression of BG enzyme in the recombinant lactic acid bacteria, wherein the promoter is operatively linked to the BG gene sequences, wherein the promoter is from a gene that encodes an antimicrobial peptide, a lantibiotic, preferably nisin gene promoter, nisA. The references of Somkuti et al., Herman et al. VanBelkum et al. and Lee et al. as applied to claims 1 and 3-5, 10-13, 25-30, drawn to a recombinant lactic acid bacteria such as *L.lactis* transformed with a BG gene, preferably the BG gene of *S.thermophilus* linked operatively to a "nar" promoter which is induced by nitrate slats under anaerobic conditions and can express very high levels of BG (up to 36,000MU) of enzyme, and permeabilization and immobilization of such recombinant *L.lactis*, has been discussed above. The promoter as taught by Lee et al. is induced by nitrate salts under anaerobic conditions. However, the use of "nar" promoter may not be amenable in processing methods of milk and milk products which is aimed for human consumption. Furthermore, the extra amounts of nitrate that remain in the product will have to be removed which calls for an extra step in the manufacture and increase the cost of production.

Kuipers et al. teach a method for controlled gene expression in lactic acid bacteria. Specifically the reference teaches the method of gene expression in any lactic acid bacteria by providing a DNA fragment which is under the control of a promoter for a microbial gene which codes for an antimicrobial peptide, which is nisA promoter of the nisin gene from *L.lactis*. The reference teaches several advantages of nisA promoter in comparison to other promoters. Unlike several other promoters, the reference teaches that promoters which code for

antimicrobial peptides, and in particular nisA promoter and nisZ promoter can be induced by their own gene products and/or derivatives thereof. The reference teaches that nisA promoter can be "strictly and absolutely" induced by nisin or a suitable analogue by means of which it is possible to induce the expression of a homologous or a heterologous gene operably linked to the said promoter in a highly controlled manner (see col.4). Furthermore, the reference also teaches that advantageously, while the induction of the preferred gene can be obtained as a positive regulation, only very small amounts i.e., less than 1 microgram/liter of the inducing factor will be required (see column 6) which does not greatly alter the food/milk composition. The antimicrobial activity of nisin is also another added advantage which kills the growth of any opportunistic contaminant in milk. The reference teaches a method of production of heterologous proteins as well as method of preparation of dairy products using the expression system in any lactic acid bacteria (see the entire document). However, the reference does not teach the use of recombinant BG gene under the control of a promoter such as nisA of nisin or that the BG produced by the permeabilized cells are of the order of 4,000-10,000 MU.

Therefore it would have been obvious to one of ordinary skill in the art to replace the "nar" promoter taught by Lee et al. in the previous paragraphs with the nisA promoter of nisin gene. One of ordinary skill in the art would have been motivated to do so as Kuipers et al. teach that a highly controlled induction of the heterologous protein, using very small amounts of nisin can be obtained with nisA promoter. One of ordinary skill in the would also be motivated to use nisA promoter as it would not add the extra step of removing the "nar" inducer "nitrate" during the manufacture of milk and milk product. One of ordinary skill in the art would have a reasonable expectation of success as Kuipers et al. demonstrate the over expression of two gene

products, beta-glucuronidase and aminopeptidase, using the nisA promoter and very small amounts of nisin as an inducer.

Therefore the above invention would have been *prima facie* obvious to one of ordinary skill in the art.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Manjunath Rao whose telephone number is (703) 306-5681. The Examiner can normally be reached on M-F from 7:30 a.m. to 4:00 p.m. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, P.Achutamurthy, can be reached on (703) 308-3804. The fax number for Official Papers to Technology Center 1600 is

(703) 305-3014. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Manjunath N. Rao Ph.D.
3/25/02